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(54) Title: ANTIMICROBIAL-PROTEIN-PRODUCING ENDOSYMBIOTIC MICROORGANISMS

(57) Abstract

There is provided a method of producing an antimicrobial-protein-producing microorganism capable of entering into an endosymbiotic relationship with a plant host comprising the combination of genetic material encoding a plant-derived antimicrobial protein with an endophyte. Examples of potent plant-derived antimicrobial proteins are given. A method for protecting a plant host from disease comprising treating the plant host with the antimicrobial-protein-producing microorganism is described.

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ANTIMICROBIAL-PROTEIN-PRODUCING  
ENDOSYMBIOTIC MICRO-ORGANISMS

This invention relates to endosymbiotic micro-organisms having the ability to produce plant-derived antimicrobial proteins.

In this context, 'antimicrobial' proteins are defined as proteins possessing at least one of the following activities: antifungal activity (which may include anti-yeast activity); antibacterial activity. Activity includes a range of antagonistic effects resulting in partial inhibition or death. 'Plant-derived' proteins are capable of being isolated from the seed or other parts of one or more plant species.

Various proteins with antimicrobial activity have been isolated from plant sources, and such proteins are often believed to take part in host defence mechanisms directed against invading or competing micro-organisms. Some of the proteins are well-characterised, and their amino acid sequence may be known. In some cases, the cDNA or gene encoding the protein has also been isolated and sequenced.

To keep out potential invaders, plants produce a wide array of antifungal compounds, either in a constitutive or an inducible manner. Several classes of proteins with antifungal properties have now been identified, including chitinases, beta-1,3-glucanases, ribosome-inactivating proteins, thionins, chitin-binding lectins and zeamatin. These proteins have gained considerable attention as they could potentially be used as biocontrol agents.

The chitinases (Schlumbaum et al, 1986, Nature, 324, 363-367) and beta-1,3-glucanases have weak activities by themselves, and are only inhibitory to plant pathogens when applied in combination (Mauch et al, 1988, Plant Physiol, 88, 936-942). The chitin-binding lectins can also be classified as rather weak antifungal factors (Broekaert et al, 1989, Science, 245, 1100-1102; Van Parijs et al, 1991, Planta, 183, 258-264). Zeamatin is a more potent antifungal protein but its activity is strongly reduced by the presence of ions at physiological concentrations (Roberts and Seliternikoff, 1990, G Gen Microbiol, 136, 2150-2155). Permatins are also known plant antifungal proteins (Vigers et al, 1991, Molec Plant-Microbe Interact, 4, 315-323; Woloshuk et al, 1991, Plant Cell, 3, 619-628). Finally, thionins (Apel et al, 1990, Physiol Plant, 80, 315-321) and ribosome-inactivating proteins (Roberts and Selitrennikoff, 1986, Biosci Rep, 6, 19-29; Leah et al, 1991, J Biol Chem, 266, 1564-1573) have antifungal activity and are known to be toxic for human cells (Carrasco et al, 1981, Eur J Biochem, 116, 185-189; Vernon et al, 1985, Arch Biochem Biophys, 238, 18-29; Stirpe and Barbieri, 1986, FEBS Lett, 195, 1-8).

Other groups of potent antimicrobial proteins with broad spectrum activity against plant pathogenic fungi (and often some antibacterial activity) are capable of isolation from certain plant species. We have previously described the structural and antifungal properties of several such proteins, including:

the small-sized cysteine-rich proteins Mj-AMP1 (antimicrobial protein 1) and Mj-AMP2 occurring in

seeds of Mirabilis jalapa (Cammue BPA et al, 1992, J Biol Chem, 267:2228-2233; International Application Publication Number WO92/15691 published on 17 September 1992);

Ac-AMP1 and Ac-AMP2 from Amaranthus caudatus seeds (Broekaert WF et al, 1992, Biochemistry, 37:4308-4314; International Application Publication Number WO92/21699 published on 10 December 1992);

Ca-AMP1 from Capsicum annuum, Bm-AMP1 from Briza maxima and related proteins found in other plants including Delphinium, Catapodium, Baptisia and Microsensis species (International Patent Application Number PCT/GB93/02179 filed on 22 October 1993);

Rs-AFP1 (antifungal protein 1) and Rs-AFP2 from seeds of Raphanus sativus (Terras FRG et al, 1992, J Biol Chem, 267:15301-15309) and related proteins such as Bn-AFP1 and Bn-AFP2 from Brassica napus, Br-AFP1 and Br-AFP2 from Brassica rapa, Sa-AFP1 and Sa-AFP2 from Sinapis alba, At-AFP1 from Arabidopsis thaliana, Dm-AMP1 and Dm-AMP2 from Dahlia merckii, Cb-AMP1 and Cb-AMP2 from Cnicus benedictus, Lc-AFP from Lathyrus cicera, Ct-AMP1 and Ct-AMP2 from Clitoria ternatea (International Patent Application Publication Number WO93/05153 published 18 March 1993);

Rs-nsLTP (non-specific lipid transfer protein) from Raphanus sativus (International Patent Application Publication Number WO93/05153 published 18 March 1993).

These publications are specifically incorporated herein by reference.

These and other plant-derived antimicrobial proteins are useful as fungicides or antibiotics to improve the disease-resistance or disease-tolerance

of crops either during the life of the plant or for post-harvest crop protection. The proteins may be extracted from plant tissue or produced by expression within micro-organisms. Exposure of a plant pathogen to an antimicrobial protein may be achieved by application of the protein to plant parts using standard agricultural techniques (eg surface spraying). The proteins may also be used to combat fungal or bacterial disease by expression within plant bodies (rather than just at the surface). DNA encoding the antimicrobial proteins (which may be a cDNA clone, a genomic DNA clone or DNA manufactured using a standard nucleic acid synthesiser) may be transformed into a plant, and the proteins expressed within transgenic plants.

It is an object of the present invention to provide an alternative method to deliver the plant-derived antimicrobial protein to its desired site of action. Such a method should be generally applicable to a wide range of plant species and may be easier or more effective than other methods.

Certain micro-organisms have the ability to enter into non-pathogenic endosymbiotic relationships with a plant host. These naturally-occurring micro-organisms, hereinafter called 'endophytes', are capable of infecting the plant host and being harboured within the plant but create no visible manifestations of disease. Such organisms include mutualistic and commensalistic endophytic organisms. The range of endophytes also includes organisms which can exist in the vascular tissues of the plant and organisms which can exist within the intercellular spaces of the plant.

A method of endophyte-enhanced protection of plants has been described in a series of patent applications by Crop Genetics International Corporation, which are discussed below and incorporated specifically herein by reference.

International Application Publication Number WO90/13224 (published 15 November 1990) describes the introduction of an endophytic bacterium into a commercially-valuable plant (such as tobacco, potato, muskmelon) to enhance protection against disease (such as tobacco mosaic virus (TMV), Pseudomonas syringae pv. tabaci, Clavibacter michiganese subsp. michiganese, potato virus X and Y, Fusarium sp. and other vascular wilt fungi). The endophyte is preferably Clavibacter xyli subsp. cynodontis (Cxc). The endophyte may be introduced into the plant by several methods including impregnating the seed with a suspension of the endophyte, using a seed coating, injecting the plant, and using a soil or foliar drench.

The endophyte may be unmodified, genetically modified (as discussed below) or formulated with other components to provide additional beneficial properties.

The endophyte may be genetically modified to produce agricultural chemicals. In this case, genetic material is derived from an agricultural-chemical-producing micro-organism and combined with a suitable endophyte. Combination of genetic material is achieved by:

- (a) forming a fusion hybrid between an endophytic bacterium and an agricultural-chemical-producing bacterium (European Patent

- Publication Number EP-125468-B1, published 28 October 1992); or
- (b) the use of recombinant techniques (insertion of DNA encoding an agricultural chemical); for example, transforming the endophyte with an expression vector which directs production of an agricultural chemical (International Application Publication Number WO91/10363, published 24 July 1991 and International Application Publication Number WO87/03303, published 4 June 1987).

Use of the modified endophyte can improve the disease tolerance of a plant host (when compared to direct application of the agrochemical or agrochemical-producing-bacterium). The endophyte may be further improved by additional genetic modification using natural or artificial techniques (such as mutagenesis). For example, the endophyte may be modified to excrete the agricultural chemical in a particular form.

The source of DNA encoding the agricultural chemical is a suitable micro-organism. Such agricultural-chemical-producing micro-organisms are described in Table I (page 27) of International Application Publication Number WO91/10363 and include a wide variety of micro-organisms producing antibiotics, antifungal agents, antibacterial agents, antiviral agents, insecticides, nematocides, miticides, herbicides, fertilisers (nitrogen-fixing or phosphate solubilising agents), plant growth regulators or anti-feeding agents.

Suitable endophytes include Agrobacterium tumefaciens, Erwinia carotovora, Pseudomonas solanacearum, Pseudomonas syringae, Xanthomonas



campestris, Streptomyces ipomoea for dicotyledonous plants; Erwinia stewartii, Xanthomonas campestris, Azospirillum lipoferum, Azospirillum brasilense, Pseudomonas syringae for monocotyledonous plants. Clavibacter xyli subsp. xyli and Clavibacter xyli subsp. cynodontis (Cxc) are particularly useful for grasses such as maize, sorghum and the like.

The agricultural-chemical-producing endophytes may be used to enhance disease protection in any plant, including those producing fruit, vegetables and flowers, trees, field and row plants such as corn, sorghum, wheat, barley, oats, rice, brome grass, sugar cane, cotton, potatoes, tomatoes, cabbage, cauliflower, broccoli, melons, cucumbers.

International Application Publication Number WO88/09114 (published 1 December 1988) describes plants colonised by beneficial endophytic micro-organisms obtained by germination of seeds impregnated with the endophytes. The endophyte may be a strain of the genus Clavibacter or Rhizobium, and may be genetically modified to produce an agricultural chemical. The seed may be from the Gramineae, Leguminosae or Halvaceae family. International Application Publication Number WO91/11907 (published 22 August 1991) describes the production of modified seed (particularly rice) containing an unmodified or modified endophyte (particularly Cxc) to produce a plant of reduced stature.

Crop Genetics International have already developed a corn bioinsecticide based upon this endophyte technology (trademark: INCIDE Technology). The INCIDE bioinsecticide consists of the endophyte

Clavibacter xyli subsp. cynodonits (Cxc) which has been genetically modified with an endotoxin gene derived from the bacterium Bacillus thuringiensis, and thus expresses a protein which is toxic to certain insect larvae. If corn seed is inoculated with the INCIDE vaccine, the modified Cxc inhabits the vascular tissue of plants grown from this seed and the crop is protected from attack by cornborer larvae. However, there may be an associated yield reduction in certain crop species or varieties (Agrow, 13/11/92, no 172, p 6).

European Patent Application Publication Number 185005 (Monsanto Co, published 18 June 1986) also describes a "plant-colonizing micro-organism" (herein called an endophyte) which has been genetically modified to express a B thuringiensis protein.

When using an agricultural-chemical-producing endophyte to enhance disease protection in a plant, the source of DNA encoding the agricultural chemical is a suitable micro-organism. Plant-derived DNA sequences encoding antimicrobial proteins have not previously been used to modify the endophytes.

To improve disease-resistance or disease-tolerance of crops, plant-derived antimicrobial proteins may be produced within the crop plant by expression of a gene incorporated into the plant genome. This may involve over-expression of an inherent protein or expression of a protein derived from another plant species. We now provide the means to express the antimicrobial protein within the crop plant without requiring plant transformation.

According to the invention, there is provided a method of producing antimicrobial-protein-producing micro-organisms capable of entering into endosymbiotic relationships with a plant host comprising the combination of genetic material encoding a plant-derived antimicrobial protein with an endophyte.

There is further provided antimicrobial-protein-producing micro-organisms produced according to the method of the invention, and seed and plants treated with said micro-organisms. Antimicrobial protein may thus be expressed within the plant by an endophyte rather than being directly expressed by the host crop plant.

As noted above, use of a genetically modified endophyte to deliver an agricultural chemical (including antifungal agents) has been described. However, the agricultural chemical was expressed from a gene derived from another micro-organism (usually a bacterium). Genes encoding plant-derived antimicrobial proteins have not been previously used (or suggested) to modify the endophyte.

Examples of plants which may be protected using the antimicrobial-protein-producing micro-organisms include field crops, cereals, fruit and vegetables such as: canola, oil seed rape, sunflower, tobacco, sugarbeet, cotton, soya, maize, wheat, barley, rice, sorghum, tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, melons, potatoes, carrot, lettuce, cabbage, onion.

DNA encoding any plant-derived antimicrobial

protein may be used in the method according to the invention (for example, DNA encoding chitinases, hevein, lectins, thionins, etc).

By way of example only, DNA encoding the following plant-derived antimicrobial proteins may be used in the method according to the invention: Mj-AMP1, Mj-AMP2, Ac-AMP1, Ac-AMP2, Ca-AMP1, Bm-AMP1, Rs-AFP1, Rs-AFP2, Br-AFP1, Br-AFP2, Bn-AFP1, Bn-AFP2, Sa-AFP1, Sa-AFP2, At-AFP1, Dm-AMP1, Dm-AMP2, Cb-AMP1, Cb-AMP2, Lc-AFP, Ct-AMP1, Ct-AMP2, Rs-nsLTP. These proteins show a high level and wide spectrum of antifungal activity, and will be particularly useful for improving disease-resistance or disease-tolerance in crops. In particular, one or more of these potent antimicrobial proteins may be used in conjunction with a slower-growing endophyte as a relatively low dose of the highly active protein may be needed to provide disease protection. The presence of a slower-growing endophyte may result in less diversion of the host plant's metabolic resources, maintaining crop yield. In addition, use of these potent plant-derived antimicrobial proteins may extend the range of plant hosts most suitable as targets for this type of disease protection. Even endophytes which are relatively poor colonisers of certain plant species (such as Cxc on wheat) may be engineered to express one or more of the potent proteins to give the desired level of protection to the host plant.

The invention will now be described by way of example only, with reference to the Sequence Listing in which:

SEQ ID NO:1 is the amino acid sequence of

Mj-AMP1.

SEQ ID NO:2 is the amino acid sequence of Mj-AMP2.

SEQ ID NO:3 is the nucleotide sequence of Mj-AMP1.

SEQ ID NO:4 is the amino acid sequence of Mj-AMP1 deduced from SEQ ID NO:3.

SEQ ID NO:5 is the nucleotide sequence of Mj-AMP2.

SEQ ID NO:6 is the amino acid sequence of Mj-AMP2 deduced from SEQ ID NO:5.

SEQ ID NO:7 is the amino acid sequence of Ac-AMP1.

SEQ ID NO:8 is the amino acid sequence of Ac-AMP2.

SEQ ID NO:9 is the nucleotide sequence of Ac-AMP2.

SEQ ID NO:10 is the amino acid sequence of Ac-AMP2 deduced from SED ID NO:9.

SEQ ID NO:11 is the amino acid sequence of Ca-AMP1.

SEQ ID NO:12 is one possible predicted DNA sequence for the Ca-AMP1 gene.

SEQ ID NO:13 is the amino acid sequence of Bm-AMP1.

SEQ ID NO:14 is one possible predicted DNA sequence for the Bm-AMP1 gene.

SEQ ID NO:15 is the amino acid sequence of Rs-AFP1.

SEQ ID NO:16 is the amino acid sequence of Rs-AFP2.

SEQ ID NO:17 is the amino acid sequence of Br-AFP1.

SEQ ID NO:18 is the amino acid sequence of Br-AFP2.

SEQ ID NO:19 is the amino acid sequence of

Bn-AFP1.

SEQ ID NO:20 is the amino acid sequence of Bn-AFP2.

SEQ ID NO:21 is the amino acid sequence of Sa-AFP1.

SEQ ID NO:22 is the amino acid sequence of Sa-AFP2.

SEQ ID NO:23 is the amino acid sequence of At-AFP1.

SEQ ID NO:24 is the amino acid sequence of Dm-AMP1.

SEQ ID NO:25 is the amino acid sequence of Dm-AMP2.

SEQ ID NO:26 is the amino acid sequence of Cb-AMP1.

SEQ ID NO:27 is the amino acid sequence of Cb-AMP2.

SEQ ID NO:28 is the amino acid sequence of Lc-AFP.

SEQ ID NO:29 is the amino acid sequence of Ct-AMP1.

SEQ ID NO:30 is the amino acid sequence of Rs-nsLTP.

SEQ ID NO:31 is one possible predicted DNA sequence for the Dm-AMP1 gene.

SEQ ID NO:32 is one possible predicted DNA sequence for the Dm-AMP2 gene.

SEQ ID NO:33 is one possible predicted DNA sequence for the Cb-AMP1 gene.

SEQ ID NO:34 is one possible predicted DNA sequence for the Cb-AMP2 gene.

SEQ ID NO:35 is one possible predicted DNA sequence for the Lc-AFP gene.

SEQ ID NO:36 is one possible predicted DNA sequence for the Ct-AMP1 gene.

SEQ ID NO:37 is the full length cDNA sequence

of Rs-AFP1.

SEQ ID NO:38 is the amino acid sequence of Rs-AFP1 deduced from SEQ ID NO:37.

SEQ ID NO:39 is the truncated cDNA sequence of Rs-AFP2.

SEQ ID NO:40 is the amino acid sequence of Rs-AFP2 deduced from SEQ ID NO:39.

SEQ ID NO:41 is the full length DNA sequence of PCR assisted site directed mutagenesis of Rs-AFP2.

SEQ ID NO:42 is the amino acid sequence of Rs-AFP2 deduced from SEQ ID NO:41.

#### EXAMPLE 1

Expression of Raphanus sativus Antifungal Protein 2 (Rs-AFP2) by the endophyte Clavibacter xyli subsp. cynodontis (Cxc).

The Rs-AFP2 protein is expressed in a system analogous to that which is known to express the Bacillus thuringiensis endotoxin. An oligonucleotide sequence coding for the antifungal protein Rs-AFP2 is prepared using Cxc-compatible codons. This oligonucleotide sequence comprises appropriate restriction sites to enable it to be exchanged with the Bacillus thuringiensis endotoxin gene sequence present in the INCIDE Cxc bacterium.

Southern analysis is used to check that Cxc is transformed with the Rs-AFP2 gene. If the result is positive, the bacterium is cultured to determine whether it is capable of expressing Rs-AFP2 protein in vitro. Western analysis and antifungal assays are carried out on the fermentation products to

determine whether the protein is produced in the correctly folded form as found in the native plant. It is known that the protein loses antifungal activity when it is reduced and hence unfolded.

#### EXAMPLE 2

Protection of rice plants using  
Rs-AFP2-producing Cxc as an antifungal agent.

Cultures of Cxc which are capable of expressing Rs-AFP2 protein are used to treat rice plants by a soil drench or seed treatment method.

The rice plants are challenged with rice blast, Pyricularia oryzae and assessed for increased resistance to the pathogen over non-Cxc-infected plants. Rs-AFP2 is known to be active against P oryzae in in vitro tests.



SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: ZENECA, Limited
- (ii) TITLE OF INVENTION: ANITMICROBIAL-PROTEIN-PRODUCING  
ENDOSYMBIOTIC MICRO-ORGANISMS
- (iii) NUMBER OF SEQUENCES: 42
- (iv) CORRESPONDENCE ADDRESS:
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  - (D) STATE: HERTFORDSHIRE
  - (E) COUNTRY: UNITED KINGDOM
  - (F) ZIP: AL7 1HD
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
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  - (A) APPLICATION NUMBER: GB 9300281.4
  - (B) FILING DATE: 08-JAN-1993
- (viii) ATTORNEY/AGENT INFORMATION:
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- (ix) TELECOMMUNICATION INFORMATION:
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  - (B) TELEFAX: 44 707 337454
  - (C) TELEX: 94028500 ICIC G

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 37 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gln Cys Ile Gly Asn Gly Gly Arg Cys Asn Glu Asn Val Gly Pro Pro  
1 5 10 15

Tyr Cys Cys Ser Gly Phe Cys Leu Arg Gln Pro Gly Gln Gly Tyr Gly  
20 25 30

Tyr Cys Lys Asn Arg  
35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Ile Gly Asn Gly Gly Arg Cys Asn Glu Asn Val Gly Pro Pro Tyr  
1 5 10 15

Cys Cys Ser Gly Phe Cys Leu Arg Gln Pro Asn Gln Gly Tyr Gly Val  
20 25 30

Cys Arg Asn Arg  
35

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 360 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTTCCCGTTG CCTTCCTCAA ATTCGCTATT GTGTTGATTC TCTTCATTGC CATGTCCGCA

ATGATAGAAG CACAATGCAT AGGAAATGGA GGAAGATGTA ACGAGAACGT GGGGCCACCA 120  
 TACTGCTGCT CCGGTTTCTG CCTCCGTCAA CCTGGACAAG GTTATGGATA TTGTAAGAAC 180  
 CGCTGAGCAA GAGCATGAAA GCAAGGCCAA TGTGTGGTCT ACTAATTTAG CCTCAAATGT 240  
 TATTTATTTG CATGTCTTGT GTTTCTTAAT TACCTTCTTT GTGTCTAAGA AGGTATAGAT 300  
 CAATAGTTTC TACTTTACTA CTATGAATAA GAGGCTTTGA TTTGGTTTAA AAAAAAAAAA 360

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Pro Val Ala Phe Leu Lys Phe Ala Ile Val Leu Ile Leu Phe Ile  
 1 5 10 15  
 Ala Met Ser Ala Met Ile Glu Ala Gln Cys Ile Gly Asn Gly Gly Arg  
 20 25 30  
 Cys Asn Glu Asn Val Gly Pro Pro Tyr Cys Cys Ser Gly Phe Cys Leu  
 35 40 45  
 Arg Gln Pro Gly Gln Gly Tyr Gly Tyr Cys Lys Asn Arg  
 50 55 60

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 433 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATATCATTCA AATATACTAA ACTAATTATA AAAAATGGCT AAGGTTCCAA TTGCCTTTCT 60  
 CAAATTCGTC ATCGTGTTGA TTCTCTTCAT TGCCATGTCA GGCATGATAG AAGCATGCAT 120

```

AGGAAATGGA GGAAGATGTA ACGAGAACGT GGGCCACCA TACTGCTGTT CGGGTTTCTG      180
CCTCCGTCAA CCTAACCAAG GTTACGGTGT TTGCAGGAAC CGCTAATAAG CAAAGCCCCAA      240
AGTGTGGGTC ACAAATAGT AGAGTTTAGC CTCAAATGTG GTTTATATAT GTAACAATCT      300
TATATGTGTT TCTCTGTGT TTCTTAATTA CCTTCTTTGT GTCTAAGAAG GTATGGATAA      360
ATAGTTTGTA CTTTACTATT ATGGTTTTTT CTTATATCAA TAAGAGGCTT TAATTAAAAA      420
AAAAAAAAAA AAA                                                                433

```

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 63 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Ala Lys Val Pro Ile Ala Phe Leu Lys Phe Val Ile Val Leu Ile
 1             5             10             15
Leu Phe Ile Ala Met Ser Gly Met Ile Glu Ala Cys Ile Gly Asn Gly
          20             25             30
Gly Arg Cys Asn Glu Asn Val Gly Pro Pro Tyr Cys Cys Ser Gly Phe
          35             40             45
Cys Leu Arg Gln Pro Asn Gln Gly Tyr Gly Val Cys Arg Asn Arg
          50             55             60

```

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Val Gly Glu Cys Val Arg Gly Arg Cys Pro Ser Gly Met Cys Cys Ser

```

19

1	5	10	15
Gln	Phe Gly Tyr Cys Gly Lys Gly	Pro Lys Tyr Cys Gly	
	20	25	

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val	Gly	Glu	Cys	Val	Arg	Gly	Arg	Cys	Pro	Ser	Gly	Met	Cys	Cys	Ser
1				5				10						15	
Gln	Phe	Gly	Tyr	Cys	Gly	Lys	Gly	Pro	Lys	Tyr	Cys	Gly	Arg		
			20				25						30		

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 590 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAAAAAAAAA	AAATAAGTC	AAGAGTATTA	ATTAGGTGAG	AAAAAATGGT	GAACATGAAG	60
TGTGTTGCAT	TGATAGTTAT	AGTTATGATG	GCGTTTATGA	TGGTGGATCC	ATCAATGGGA	120
GTGGGAGAAT	GTGTGAGAGG	ACGTTGCCCA	AGTGGGATGT	GTTGCAGTCA	GTTTGGGTAC	180
TGTGGTAAAG	GCCCAAAGTA	CTGTGGCCGT	GCCAGTACTA	CTGTGGATCA	CCAAGCTGAT	240
GTTGCTGCCA	CCAAAACTGC	CAAGAATCCT	ACCGATGCTA	AACTTGCTGG	TGCTGGTAGT	300
CCATGAAAGT	AGTAGTAGC	TAGGTTACAG	TTGGATTACC	AAGCCGTGCC	AGTACTACTG	360
TGGCCGTGCC	AGTACTAATG	TTCTCTTATA	TGTCTGAAAT	AAGCTCCTAT	ATAAATACTA	420
GTATCTTGAT	GTAATGGAGT	ATTTTCATTT	TGTTTTTATT	TGAGTTATGA	TCGTGACTTC	480

CTTGTGTTGG TTAACTTGT ATATTGTAAT GCATCTTAAA TGCTGTCTCA AATAATTGA 540  
 TGTATTAAAC ACTTGTTTTG TTTTAATAC ATACTAAGTG CTGTAAATTC 590

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Val Asn Met Lys Cys Val Ala Leu Ile Val Ile Val Met Met Ala  
 1 5 10 15  
 Phe Met Met Val Asp Pro Ser Met Gly Val Gly Glu Cys Val Arg Gly  
 20 25 30  
 Arg Cys Pro Ser Gly Met Cys Cys Ser Gln Phe Gly Tyr Cys Gly Lys  
 35 40 45  
 Gly Pro Lys Tyr Cys Gly Arg Ala Ser Thr Thr Val Asp His Gln Ala  
 50 55 60  
 Asp Val Ala Ala Thr Lys Thr Ala Lys Asn Pro Thr Asp Ala Lys Leu  
 65 70 75 80  
 Ala Gly Ala Gly Ser Pro  
 85

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gln Glu Gln Cys Gly Asn Gln Ala Gly Gly Arg Ala Cys Ala Asn Arg  
 1 5 10 15

Gly Val Gly Cys Gln Ser Asn Cys Gly Arg  
35 40

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 126 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

(2) INFORMATION FOR SEQ ID NO:13:

(ii) MOLECULE TYPE: protein

(A) NAME/KEY: Modified-site  
(B) LOCATION: 15  
(D) OTHER INFORMATION: /note= "Xaa at position 15 may be R  
or H"

(A) NAME/KEY: Modified-site  
(B) LOCATION: 29  
(D) OTHER INFORMATION: /note= "Xaa at position 29 may be S  
or N"

Cys Ser Ser His Asn Pro Cys Pro Arg His Gln Cys Cys Ser Xaa Tyr  
1 5 10 15

Gly Tyr Cys Gly Leu Gly Ser Asp Tyr Cys Gly Leu Xaa Cys Arg Gly  
 20 25 30

Gly Pro Cys Asp Arg  
 35

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGCTCTTCTC ACAACCCGTG CCCGAGACAC CAATGCTGCT CTAAGTACGG ATACTGCGGA 60  
 CTTGGATCTG ACTACTGCGG ACTTGGATGC AGAGGAGGAC CGTGCGACAG A 111

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
 1 5 10 15  
 Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Asn Leu Glu Lys Ala Arg  
 20 25 30  
 His Gly Ser Cys Asn Tyr Val Phe Pro Ala His Lys  
 35 40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single



(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Lys Leu Cys Gln Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
1 5 10 15

Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Arg Leu Glu Lys Ala Arg  
20 25 30

His Gly Ser Cys  
35

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
1 5 10 15

Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Asn  
20 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Xaa Ser Gly Val Cys Gly  
1 5 10 15

Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Arg  
 20 25

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
 1 5 10 15

Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Asn Leu Glu Lys  
 20 25 30

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
 1 5 10 15

Asn Asn Asn Ala Cys Lys Asn  
 20

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
 1 5 10 15

Asn Asn Asn Ala Cys Lys Asn Gln Cys  
 20 25

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Gln Lys Leu Cys Gln Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
 1 5 10 15

Asn Asn Asn Ala Cys Arg Asn Gln Cys Ile  
 20 25

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gln Lys Leu Cys Glu Arg Pro Ser Gly Thr Trp Ser Gly Val Cys Gly  
 1 5 10 15

Asn Ser Asn Ala Cys Lys Asn Gln Cys Ile Asn  
 20 25

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 amino acids
- (B) TYPE: amino acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Glu Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn  
1 5 10 15

Thr Gly His Cys Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His  
20 25 30

Gly Ala Cys His Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe  
35 40 45

Asn Cys  
50

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Glu Val Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn  
1 5 10 15

Thr Gly His Cys  
20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 50 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

(2) INFORMATION FOR SEQ ID NO:27:

(A) LENGTH: 50 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

(2) INFORMATION FOR SEQ ID NO:28:

(A) LENGTH: 47 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Lys Thr Cys Glu Asn Leu Ser Gly Thr Phe Lys Gly Pro Cys Ile Pro

28

1	5	10	15
Asp Gly Asn Cys Asn Lys His Cys Lys Asn Asn Glu His Leu Leu Ser	20	25	30
Gly Arg Cys Arg Asp Asp Phe Xaa Cys Trp Cys Thr Arg Asn Cys	35	40	45

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Asn Leu Cys Glu Arg Ala Ser Leu Thr Trp Thr Gly Asn Cys Gly Asn	1	5	10	15
Thr Gly His Cys Asp Thr Gln Cys Arg Asn Trp Glu Ser Ala Lys His	20	25	30	
Gly Ala Cys His Lys Arg Gly Asn Trp Lys Cys Phe Cys Tyr Phe Asp	35	40	45	
Cys				

## (2) INFORMATION FOR SEQ ID NO:30:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Leu Ser Cys Gly Thr Val Asn Ser Asn Leu Ala Ala Cys Ile Gly	1	5	10	15
Tyr Leu Thr Gln Asn Ala Pro Leu Ala Arg Gly Cys Cys Thr Gly Val	20	25	30	

Thr Asn Leu Asn Asn Met Ala Xaa Thr Thr Pro  
35 40

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 150 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GAGCTTTGCG AGAAGGCTTC TAAGACTTGG TCTGGAAACT GCGGAAACAC TGGACATTGC 60  
GATAACCAAT GCAAGTCTTG GGAGGGAGCT GCTCATGGAG CTTGCCATGT TAGAAACGGA 120  
AAGCATATGT GCTTCTGCTA CTTCAACTGC 150

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGGTTTGCG AGAAGGCTTC TAAGACTTGG TCTGGAAACT GCGGAAACAC TGGACATTGC 60

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 150 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAGCTTTGCG AGAAGGCTTC TAAGACTTGG TCTGGAAACT GCGGAAACAC TAAGCATTGC 60  
GATGATCAAT GCAAGTCTTG GGAGGGAGCT GCTCATGGAG CTTGCCATGT TAGAAACGGA 120  
AAGCATATGT GCTTCTGCTA CTTCAACTGC 150

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 150 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GAGCTTTGCG AGAAGGCTTC TAAGACTTGG TCTGGAAACT GCGGAAACAC TAAGCATTGC 60  
GATAACAAGT GCAAGTCTTG GGAGGGAGCT GCTCATGGAG CTTGCCATGT TAGATCTGGA 120  
AAGCATATGT GCTTCTGCTA CTTCAACTGC 150

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 141 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AAGACTTGCG AGAACCTTTC TGGAACCTTC AAGGGACCAT GCATTCCAGA TGGAAACTGC 60  
AACAAGCATT GCAAGAACAA CGAGCATCTT CTTTCTGGAA GATGCAGAGA TGATTTCNNN 120  
TGCTGGTGCA CTAGAAACTG C 141

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 147 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear



(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AACCTTTGCG AGAGAGCTTC TCTTACTTGG ACTGGAAACT GCGGAAACAC TGGACATTGC	60
GATACTCAAT GCAGAAACTG GGAGTCTGCT AAGCATGGAG CTTGCCATAA GAGAGGAAAC	120
TGGAAGTGCT TCTGCTACTT CGATTGC	147

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 414 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTTTATTAG TGATCATGGC TAAGTTTGCG TCCATCATCG CACTTCTTTT TGCTGCTCTT	60
GTTCTTTTTG CTGCTTTCGA AGCACCAACA ATGGTGGAAG CACAGAAGTT GTGCGAAAGG	120
CCAAGTGGGA CATGGTCAGG AGTCTGTGGA AACAATAACG CATGCAAGAA TCAGTGCATT	180
AACCTTGAGA AAGCACGACA TGGATCTTGC AACTATGTCT TCCCAGCTCA CAAGTGTATC	240
TGCTACTTTC CTGTGTAATT TATCGCAAAC TCTTTGGTGA ATAGTTTTTA TGTAATTTAC	300
ACAAAATAAG TCAGTGTAC TATCCATGAG TGATTTTAAG ACATGTACCA GATATGTTAT	360
GTTGGTTCGG TTATACAAAT AAAGTTTTAT TCACCAAAAA AAAAAAAAAA AAAA	414

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met	Ala	Lys	Phe	Ala	Ser	Ile	Ile	Ala	Leu	Leu	Phe	Ala	Ala	Leu	Val
1				5					10					15	
Leu	Phe	Ala	Ala	Phe	Glu	Ala	Pro	Thr	Met	Val	Glu	Ala	Gln	Lys	Leu
			20					25					30		
Cys	Glu	Arg	Pro	Ser	Gly	Thr	Trp	Ser	Gly	Val	Cys	Gly	Asn	Asn	Asn
		35					40					45			
Ala	Cys	Lys	Asn	Gln	Cys	Ile	Asn	Leu	Glu	Lys	Ala	Arg	His	Gly	Ser
	50					55					60				
Cys	Asn	Tyr	Val	Phe	Pro	Ala	His	Lys	Cys	Ile	Cys	Tyr	Phe	Pro	Cys
65						70				75					80

## (2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 284 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGAAATAATA ACGCATGCAA GAATCAGTGC ATTCGACTTG AGAAAGCACG ACATGGGTCT	60
TGCAACTATG TCTTCCCAGC TCACAAGTGT ATCTGTTATT TCCCTTGTTA ATTCCATAAA	120
CTCTTCGGTG GTTAATAGTG TCGCATATT ACATATAATT AATAAGTTTG TGTCCTATT	180
TATTAGTGAC TTTATGACAT GTGCCAGGTA TGTTTATGTT GGGTTGGTTG TAATATAAAA	240
AAGTTCACGG ATAATAAGAT GATAAGCTCA CGTCGCCAAA AAAA	284

## (2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 36 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

(2) INFORMATION FOR SEQ ID NO:41:

(A) LENGTH: 288 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

(2) INFORMATION FOR SEQ ID NO:42:

(A) LENGTH: 80 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

34

35

40

45

Ala Cys Lys Asn Gln Cys Ile Arg Leu Glu Lys Ala Arg His Gly Ser  
50 55 60

Cys Asn Tyr Val Phe Pro Ala His Lys Cys Ile Cys Tyr Phe Pro Cys  
65 70 75 80

SUBSTITUTE SHEET

We claim:

1. A method of producing an antimicrobial-protein-producing micro-organism capable of entering into an endosymbiotic relationship with a plant host comprising the combination of genetic material encoding a plant-derived antimicrobial protein with an endophyte.
2. A method according to claim 1 in which the plant-derived antimicrobial protein is selected from the protein group consisting of Mj-AMP1, Mj-AMP2, Ac-AMP1, Ac-AMP2, Ca-AMP1, Bm-AMP1, Rs-AFP1, Rs-AFP2, Br-AFP1, Br-AFP2, Bn-AFP1, Bn-AFP2, Sa-AFP1, Sa-AFP2, At-AFP1, Dm-AMP1, Dm-AMP2, Cb-AMP1, Cb-AMP2, Lc-AFP, Ct-AMP1, Ct-AMP2 and Rs-nsLTP.
3. A method according to claim 1 in which the endophyte is Clavibacter xyli subsp. cynodontis.
4. An antimicrobial-protein-producing micro-organism produced by the method according to claim 1.
5. A method for protecting a plant host from disease comprising treating the plant host with the antimicrobial-protein-producing micro-organism according to claim 4.
6. A plant or seed treated with an antimicrobial-protein-producing micro-organism according to claim 4.

## INTERNATIONAL SEARCH REPORT

Int. Appl. No.

PCT/GB 94/00012

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/29 C12N15/74 A01N63/00 C12N1/21 A01H5/00  
A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N A01N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 10363 (CROP GENETICS INTERNATIONAL) 25 July 1991 see the whole document ---	1
Y	EP,A,0 474 601 (CIBA-GEIGY) 11 March 1992 see the whole document ---	1
A	EP,A,0 256 682 (ICI) 24 February 1988 see the whole document -----	1

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

14 April 1994

Date of mailing of the international search report

27 -04- 1994

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 94/00012

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9110363	25-07-91	AU-A- 7159291	05-08-91
EP-A-0474601	11-03-92	AU-B- 646492	24-02-94
		AU-A- 8372091	12-03-92
		CA-A- 2050743	08-03-92
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		JP-A- 63041410	22-02-88

